

fragment 1 of the V_H domain of anti-CD19, followed by a segment which codes for a GlyGly linker, was produced using the primers DP1,

5'-TCACACAGAATTC-TTAGATCTATTAAAGAGGAGAAATTAACC (SEQ ID NO:1)

and DP2, 5'-AGCACACGATATCACCGCCAAGCTTGGGTGTTGTTTTGGC (SEQ ID NO:2) (FIGURE. 2). The PCR fragment 1 was cleaved by EcoRI and EcoRV and ligated

with the EcoRI/EcoRV-linearized plasmid pHOG-dmOKT3 so as to produce the vector

pHOG19-3. The PCR fragment 2 of the V_L domain of anti-CD19, followed by a segment

which codes for a c-myc epitope and a hexahistidiny tail, was produced using the primers

DP3, 5'-AGCACACAAGCTTGGCGGTGATATCTTGCTCACCCAAAC-TCCA, (SEQ ID NO:3) and DP4,

5'-AGCACACTCTAGAGACACACAGATCTTTAGTGATGGTGAT-GGTGATGTGAGTT

TAGG (SEQ ID NO:4). The PCR fragment 2 was cleaved by HindIII and XbaI and ligated

with the HindIII/XbaI-linearized plasmid pHOG-dmOKT3 so as to obtain the vector pHOG3-

19 (FIGURE 2). The gene coding for the hybrid scFv-3-19 in the plasmid pHOG3-19 was

amplified by means of PCR with the primers Bi3sk,

5'-CAGCCGGCCATGGCGCAGGTGCAACTGCAGCAG (SEQ ID NO:5) and either Li-1,

5'-TATATACTGCAGCTGCACCTGGCTACCACCACCACCGGAGCCG-CCACCACCGC

TACCACCGCCGCCAGAACCACCACCACCAGCGGCCGCAGCATCAGCCCG,(SEQ ID

NO:6) for the production of a long flexible (Gly₄Ser)₄ inter-scFV linker (PCR fragment 3,

FIGURE 2) or Li-2,

5'-TATATA-CTGCAGCTGCACCTGCGACCCTGGGCCACCAGCGGCCGCAGCATCA

GCCG, (SEQ ID NO:7) for the production of a short rigid GGPGS linker (PCR fragment 4,

FIGURE 2). The expression plasmids pDISC3x19-LL and pDISC3x19-SL were constructed

by ligating the NcoI/PvuII restriction fragment from pHOG19-3, comprising the vector

framework and the NcoI/PvuII-cleaved PCR fragments 3 and 4, respectively (FIGURES 3

and 4). The complete nucleotide and protein sequences of the bivalent and tetravalent F_v

antibody constructs are indicated in FIGURES 5 and 6, respectively.

On page 9, please replace the paragraph beginning, "The vector pPICZαA (Invitrogen BV, Leek, Netherlands) for the expression and secretion of recombinant proteins..." with the following paragraph:

The vector pPICZαA (Invitrogen BV, Leek, Netherlands) for the expression and secretion of recombinant proteins in the yeast *Pichia pastoris* was used as a starting material. It contains a gene which codes for the *Saccharomyces cerevisiae* α-factor secretion signal,

followed by a polylinker. The secretion of this vector is based on the dominant selectable marker, Zeocin™ which is bifunctional in both *Pichia* and *E. coli*. The gene which codes for the tetravalent F_v antibody construct (scDia-SL) was amplified by means of PCR by the template pDIC3x19-SL using the primers 5-PIC, 5'-CCGTGAATTCAGGTGCAACTGCAGCAGTCTGGGGCTGAACTGGC, and pSEXBn (SEQ ID NO:8). 5'-GGTCGACGTTAACCGACAAACAACAGATAAAACG (SEQ ID NO:9). The resulting PCR product was cleaved by EcoRI and XbaI and ligated in EcoRI/XbaI-linearized pPICZαA. The expression plasmid pPIC-DISC-SL was obtained. The nucleotide and protein sequences of the tetravalent F_v antibody construct are shown in FIGURE 7.

On page 13, please replace the paragraph beginning, "Expression vectors were prepared which contained the hok/sok plasmid-free cell suicide system..." with the following paragraph:

Expression vectors were prepared which contained the hok/sok plasmid-free cell suicide system and a gene which codes for the *skp*/OmpH periplasmic factor for a greater production of recombinant antibodies. The *skp* gene was amplified by PCR using the primers *skp*-1, 5'-CGA ATT CTT AAG ATA AGA AGG AGT TTA TTG TGA AAA AGT GGT TAT TAG CTG CAG G (SEQ ID NO:10) and *skp*-2, 5'-CGA ATT AAG CTT CAT TAT TTA ACC TGT TTC AGT ACG TCG G (SEQ ID NO:11) using the plasmid pGAH317 (Holck and Kleppe, 1988, *Gene* 67:117-124). The resulting PCR fragment was cleaved by AflII and HindIII and inserted in the AflII/HindIII-linearized plasmid pHKK (Horn et al., 1996, *Appl. Microbiol. Biotechnol.* 46, 524-532) so as to obtain the vector pSKK. The genes obtained in the plasmids pDISC3x19-LL and pDISC3x19-SL and coding for the scFv antibody constructs were amplified by means of the primers *fe*-1, 5'-CGA ATT TCT AGA TAA GAA GGA GAA ATT AAC CAT GAA ATA CC (SEQ ID NO:12) and *fe*-2, 5'-CGA ATT CTT AAG CTA TTA GTG ATG GTG ATG GTG ATG TGA G (SEQ ID NO:13). The XbaI/AflIII-cleaved PCR-fragments were inserted in pSKK before the *skp* insert so as to obtain the expression plasmids pDISC5-LL and pDISC6-SL, respectively, which contain tricistronic operons under the control of the lac promoter/operator system (FIGURES 9 and 10).